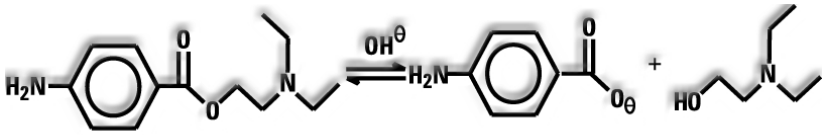
Saponification of procaine: Kinetic measurements with the Agilent high throughput analysis system

Application Note

Udo Huber



Abstract

In this application note we describe how the cleavage of procaine, a p-aminobenzoic acid ester, can be monitored using the Agilent 220 microplate sampler (MPS) with the Agilent 1100 Series LC system. The data of the measurements is transferred to ChemStore C/S, the database module of the Agilent ChemStation Plus, for data analysis. We show that the data can then be transferred easily to a spreadsheet program, for example Microsoft® Excel®, for further calculations such as determination of the rate coefficient.

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Introduction

Kinetic measurements play an important role in pharmaceutical chemistry. Not only for pharmacokinetics where the rate of active compound degradation has to be determined, but also for drug discovery to test the inhibition effect of a compound on an enzyme. For very fast reactions special apparatus, for example shock tubes, have to be used but slower reactions can be monitored by analyzing reaction samples at specific time intervals. This application note describes how this is achieved using the Agilent 220 MPS with the Agilent 1100 Series LC System and the Agilent ChemStation Plus software. Saponification of procaine at pH=10 was selected as a model scenario.

Procaine is a p-aminobenzoic acid ester, which can be saponificated into p-aminobenzoic acid (PABA) and an alcohol. The reaction is shown in figure 1.

Since the reaction is first order the rate of reaction can be described as:

$$v = \frac{d[Ester]}{dt} = -k \cdot [Ester]$$

with:

v (rate of reaction)
k (rate coefficient)
[Ester] (concentration of procaine)

Integration of this formula gives:

$$\ln \frac{[Ester]_t}{[Ester]_0} = -k \cdot t$$

The rate coefficient k can be determined from the slope of the straight line in the graph $\ln([Ester]_t/[Ester]_0)$ against time.

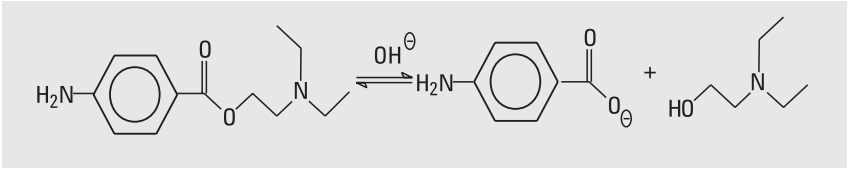

Figure 1
Saponification of procaine

Equipment

The system included an Agilent 1100 Series vacuum degasser, an Agilent 1100 Series binary pump, an Agilent 1100 Series thermostatted column compartment, an Agilent 1100 Series diode array detector and an Agilent 220 micro plate sampler.

The system was controlled using the Agilent ChemStation Plus (version A.07.01) and the micro plate sampling software (version A.03.01).

System Setup Overview

1. A chromatographic method for measuring procaine and PABA was developed on the Agilent 220 MPS and the Agilent 1100 Series LC system.

2. Standards for both compounds were measured, the method was calibrated and the run time was extended to 20 minutes (figure 2).

3. Three procaine samples were dissolved in 0.025 M NaH_2PO_4, buffer adjusted to pH=10. These samples were measured with the method described before, which gives an overall run time of one hour for the three samples.

4. The measurement was repeated 24 times to give an overall study run time of 24 hours.

5. The measured data was automatically transferred to the ChemStation Plus database module were the *Charts amount against reaction time* was created.

6. To determne the rate coefficient the data was then automatically transferred to Microsoft Excel.

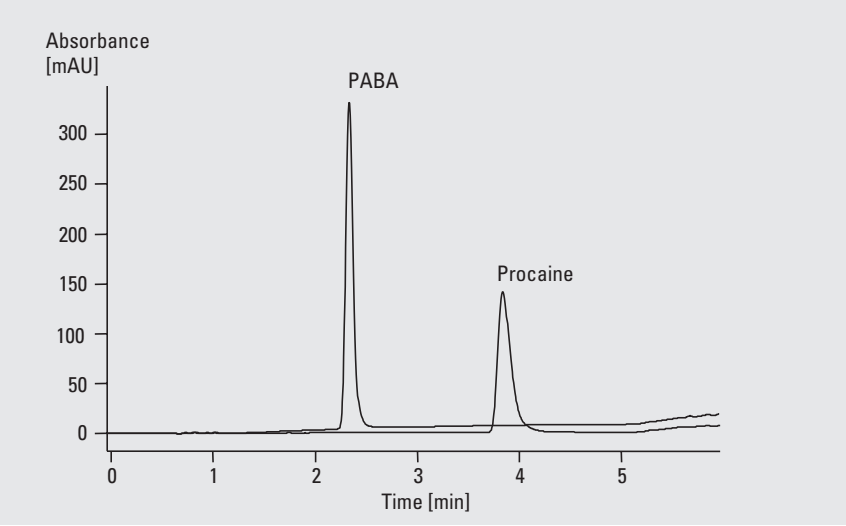

Figure 2
Measurement of standards

Mobile Phases:	A= 0.025M NaH_2PO_4 in water (pH=2.5), B = ACN
Gradient:	5 % B for 3.5 min, flow 1 ml/min 5 % B to 50 % B in 1.5 min, flow 1 ml/min 50 % B for 0.5 min, flow 1 ml/min 50 % B to 5 % B in 0.5 min, flow 1 ml/min 5 % B, flow from 1 ml/min to 0.1 ml/min in 0.1 min 5 % B, flow 0.1 ml/min for 18.9 min 5 % B, flow 0.1 ml/min to 1 ml/min in 0.1 min 5 % B for 0.9 min, flow 1 ml/min
Stop time:	20 min
Column:	Zorbax SB-C18, 4.6 x 75 mm, 5 µm
Column temp.:	50 °C
UV detector:	DAD 204 nm/16 (reference 360 nm/100)

Results and Discussion

Method calibration

A three-level calibration was done after measuring standards for procaine and PABA using the method in figure 2.

Study setup and sample measurement

The method above was renamed four times and set up in the *Study Parameters* screen. *Injection ordered by method* was selected and three samples were set up, as shown in figure 3. Since every method runs for about 20 minutes, each of the three samples was analyzed every hour. To measure the samples over 24 hours the study was repeated six times. This was set up in the *Start Study* window.

The study was started and the measured sample data was automatically transferred to a ChemStation Plus database study, which was set up before.

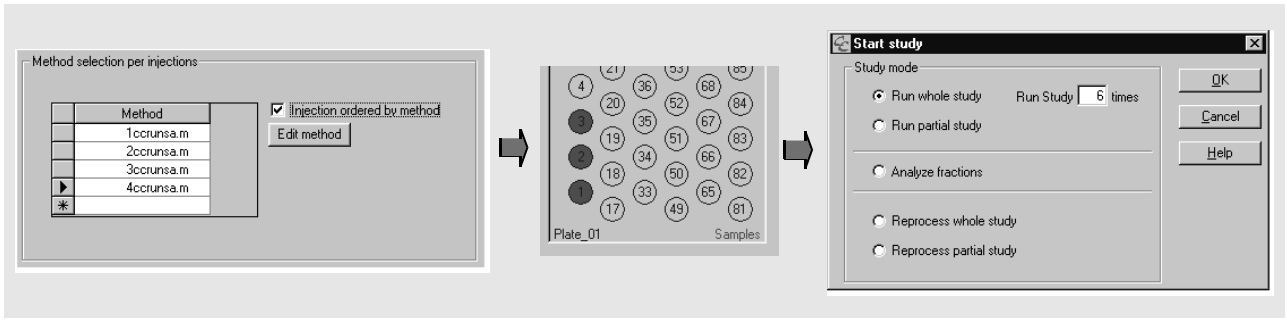

Figure 3
Study setup

ChemStation Plus database results and charts

The study results were loaded into the ChemStation Plus database module and *Sample Name*, *Injection Time* and *Amount* were displayed in *Compound* view. By selecting procaine and/or PABA in the *Compound List* the results were displayed in a comprehensible table (*Table Layout*). The results for a specific sample were displayed using a Filter on the field *Sample Name*. The reaction is first order, as can be clearly seen in the chart shown in figure 4, which was created in the *Chart Layout* view of the ChemStation database module.

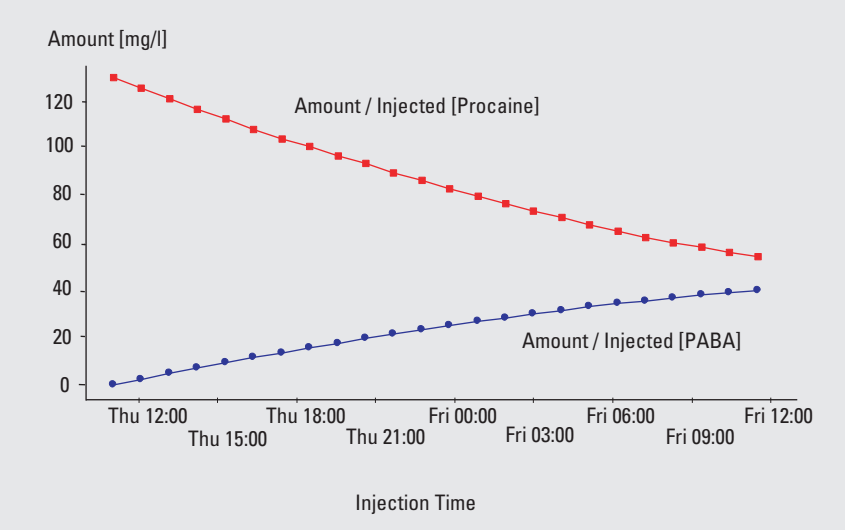

Figure 4
Saponification of procaine: Amount against reaction time

Determination of the rate coefficient by exporting the data to Microsoft Excel

The table created for procaine containing the fields *Sample Name*, *Injection Time* and *Amount* in the ChemStation Plus database module was filtered for one sample and transferred to a Microsoft Excel file. This was done using the *Export* function of the ChemStation Plus database module by selecting *Data* and *MS Excel* in the *Export* window. In Microsoft Excel the injection time difference was calculated in seconds beginning at the first injection at t_0. The calculated value gives the x-axis of figure 5.
The y-axis is calculated as $\ln([Ester]_t/[Ester]_0)$. The negative value of the rate coefficient is the slope of the resulting straight line (figure 5). The calculated results for the first seven injections are shown in table 1.

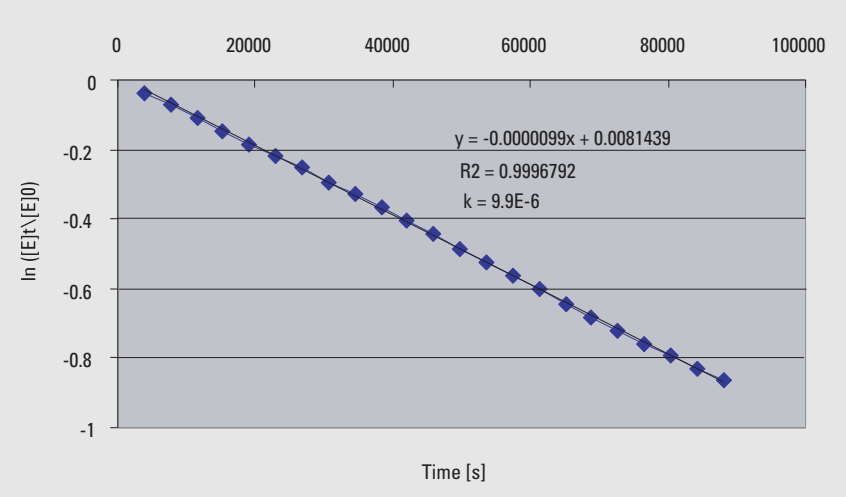

Figure 5
Determination of rate coefficient

Sample injected (X)	Time difference [hh:mm:ss]	Time difference [s]	Procaine amount (Y) [mg/l]	$\ln \frac{[Ester]_t}{[Ester]_0}$
2/3/00 11:02:59 AM	= t_0		129.838290062179	= [Ester]0
2/3/00 12:06:45 PM	1:03:46	3826.00	125.14649513062	-0.036804741
2/3/00 1:10:30 PM	2:07:31	7651.00	120.783350276534	-0.072291306
2/3/00 2:14:27 PM	3:11:28	11488.00	116.502232323668	-0.108379319
2/3/00 3:18:14 PM	4:15:15	15315.00	112.19641294469	-0.146038731
2/3/00 4:22:05 PM	5:19:06	19146.00	108.006644852136	-0.184097002
2/3/00 5:25:56 PM	6:22:57	22977.00	104.201332127358	-0.21996484

Table 1
Calculated results

Conclusion

In this application note a kinetic measurement for the saponification of procaine at pH=10 was performed and analyzed using the Agilent 220 MPS, the Agilent 1100 Series LC system and the Agilent ChemStation Plus. The progress of the reaction was monitored in the ChemStation Plus database module and the data was transferred further to Microsoft Excel for calculation of the rate coefficient. The transfer was done automatically. It was not necessary to transfer the data manually, which would have been a slow, tedious and error-prone process.

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